Antibacterial potency and differential synergistic effects of *Zingiber officinale* and *Capsicum annum* extracts against *E. coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*

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ABSTRCT

There is need to look for nontoxic cheap alternative ways of fighting mutating and resistant disease causing microbes especially in sub-Saharan region. This is because of the lower average immune system strength due to bad feeding habits, high population and increase of disease causing organisms which are exacerbating the HIV and AIDS menace. The use of species to preserve, give flavour to food and to fight illnesses has been practiced in Africa since the invasion of Asian populations in the coastal regions. In this study, we investigated the potential of using common spices like Ginger (*Zingiber officinale*) and Chilli pepper (*Capsicum annuum*) against *Escherichia coli, Pseudomonas aeruginosa* and *staphylococcus aureus* which are common bacteria mostly found in contaminated food. This was done using the Kirby Bauer diffusion method. Ginger and chili pepper were found to inhibit the growth of the tested bacteria especially *staphylococcus aureus* through comparing the diameter of inhibition zones. The results indicated that extracts of ginger and chilli pepper had antibacterial activity in the range of 15-33mm. *E. coli* and *Staphylococcus aureus* were more affected by the extracts than *Pseudomonas aeruginosa.* However, a mixture of the two extracts produced a greater antibacterial activity than the individual extracts with *p* values nearing 0.05. Therefore, the extracts displayed an antimicrobial activity with greater efficacy when acting synergistically on the test organisms. Therefore, the potential of using the combination as a naturopathy is still high despite the perceived microbial resistance after a long time of usage in human populations.

Keywords: Ginger; Chili Pepper; Resistance; Immune systems; Inhibition zone; Sub-Saharan

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INTRODUCTION

The use of medicinal plant to treat ailment associated with pains is well known through history. Such plants can play important role in drug discovery and such practice has been regarded as logical research strategy for searching new drugs (Auta et al., 2011). The acquaintance with different ethnic groups has contributed to the developed of research on natural products. This has been used to decipher many phenomenon like the close relationship between the chemical structure of a compound and its biological property in many animal-plant interrelations. Spices and herbs have been used for thousand of centuries by many cultures to enhance the favour and aroma of food. Microbial inhibitory activities of spices and their derivatives have also been identified. (Shelef et al, 1983). A number of spices have been screened for antimicrobial activity (Premanth et al, 2011) and efforts have been done to identify their active constituents. Studies have shown that both gram negative and positive food-borne and water–borne bacteria are inhibited by garlic, onion, cinnamon, cloves, thyme and sage (Auta et al, 2011).

Ginger is a member of the family Zingiberaceous, a small family with more than 45 genera and spices. Ginger is the underground rhizome of the ginger plant with a firm striated texture (Hoffman et al, 2012). On the other hand, chili pepper is a member of Solanaceae family and the capsicum species. Research has shown that it has activity against food borne pathogenic bacteria (Dasgupta et al, 2011). Ginger and chilli pepper are reputed to be effective against *E. coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* which cause significant illness in humans (Hara et al, 2006). *Zingiber officinale* has antimicrobial activity and thus can be used in treatment of bacterial infections (Tan and Vanitha et al,. 2004). This this because of plants have almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives most of which are secondary metabolites (Geissman et al,.1963). The

capsaicinoids of chili pepper have demonstrated a degree of biological activity affecting the cardiovascular and digestive system (Virus and Gebhart et al, 1979). Antimicrobial activity of chili pepper has been shown.

The use of spices and other plants as antimicrobial agents developed due to emergence of multiple antibiotic resistances among such pathogen as *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* (Wald et al, 2000). This is because of intrinsic virulence, its ability to cause a diverse array of life threatening infection and the capacity to adapt to different environmental infections (Lowry et al, 1998). *Pseudomonas aeruginosa* is not easily controlled by antibiotics because it is gram negative and has fairly impermeable outer and can also turn on pumps that remove antibiotics from the cell. Microbes attain resistance because of adaptability feature conferred by plasmids which carry integrin and gene cassettes (Kumarasamy et al, 2010).

Research by BMJ in 2015 showed that people who eat spicy foods nearly every day have 14% chance of living longer than those who consume spicy foods less than once a week .Regular spicy food eaters also are less likely to die from cancer, heart and respiratory diseases than those who eat spicy foods infrequently. Bioactive ingredients in spices have been found to have anti- obesity, antioxidant, anti-inflammation and anti-cancer properties. This research was set out to investigate the differences in microbial potency of the ginger and capsicum spices when used individually and when used in combinations, the research also examined the magnitude of the effect of the two spices on *Staphylococcus aureus and E. coli* in relation to *Pseudomonas aeruginosa*.

Methodology

The study was carried out under controlled environmental conditions at both the South-Eastern Kenya University laboratory and the institute of Biotechnology Research laboratory at Jomo Kenyatta University of Agriculture and Technology in Kenya. The plant material of *Zingiber officinale* rhizomes and *Capsicum annuum* fruits were obtained at a garden in Institute of Biotechnology Research in Nairobi.

Extraction of plant material

The ginger rhizomes were washed with distilled water and allowed to air dry for 2 days. Extraction was done using the crude extraction method using methanol as described by Fatope et al,.1993. About 40g of ginger was blended into powder and soaked into 100 ml of methanol

for 24 hours with intermediate shaking. The extract was stored in refrigerator until use. Chilli pepper fruits extracts were prepared by macerating pepper powder in methanol for 24 hours then filtered. The residue was washed twice with fresh methanol and the filtrates combined with the first filtrate. Combined filtrate was evaporated to dryness to produce sticky materials from the chili pepper extract

Culturing the test micro-organisms and their inoculation

Pure isolates of *E. coli, Pseudomonas aeruginosa* and *staphylococcus aureus* were provided by the Institute of Biotechnology Research (IBR) then maintained in nutrient agar slants and incubated to match turbidity of 0.5 according to McFarland standards. Mueller Hinton agar was prepared according to the manufacturer's instruction then dispensed into petri dishes. The agar plates containing the Mueller Hinton Agar medium was inoculated with 0.01ml of the inoculum using an inoculating loop and spread using a spreader. Assay discs around 6mm diameter were soaked on the extracts then transferred on the media surface to determine the antibacterial activity according to Bauer AW *et al,* 1966. All plates were incubated at 37^0C for 15 hours and the zones of inhibition measured using a ruler after 15 hours and 30 hours. The experiment was carried out in triplicates and for the control the disks, pure methanol was used since the extraction was done using methanol.

Statistical analysis

The results were expressed as means of diameter of zones of inhibition in millimeters at 15 hours and 30 hours and calculation done using Microsoft excel and R software where Welch Two Sample t-test was done to determine the significant time. Graphs were included to illustrate and compare the inhibitory and synergetic effects of the spice extracts.

RESULTS

Comparing the inhibition zones

The methanolic extracts of ginger, chili pepper and combined ginger and chili pepper plants material showed antimicrobial activity on the tested organisms. The highest susceptibility was recorded with the combined ginger + chili pepper extract followed by extracts of chili pepper then

ginger extracts. (Figure 1) The antibacterial efficacy of the mixture was highest compared to the individual extracts. The control didn't have zones of inhibition hence did not show antibacterial activity. The *p* values for the comparisons was > 0.05 hence not significant with the ginger + chili pepper mixture which had a *p* value close to 0.05.

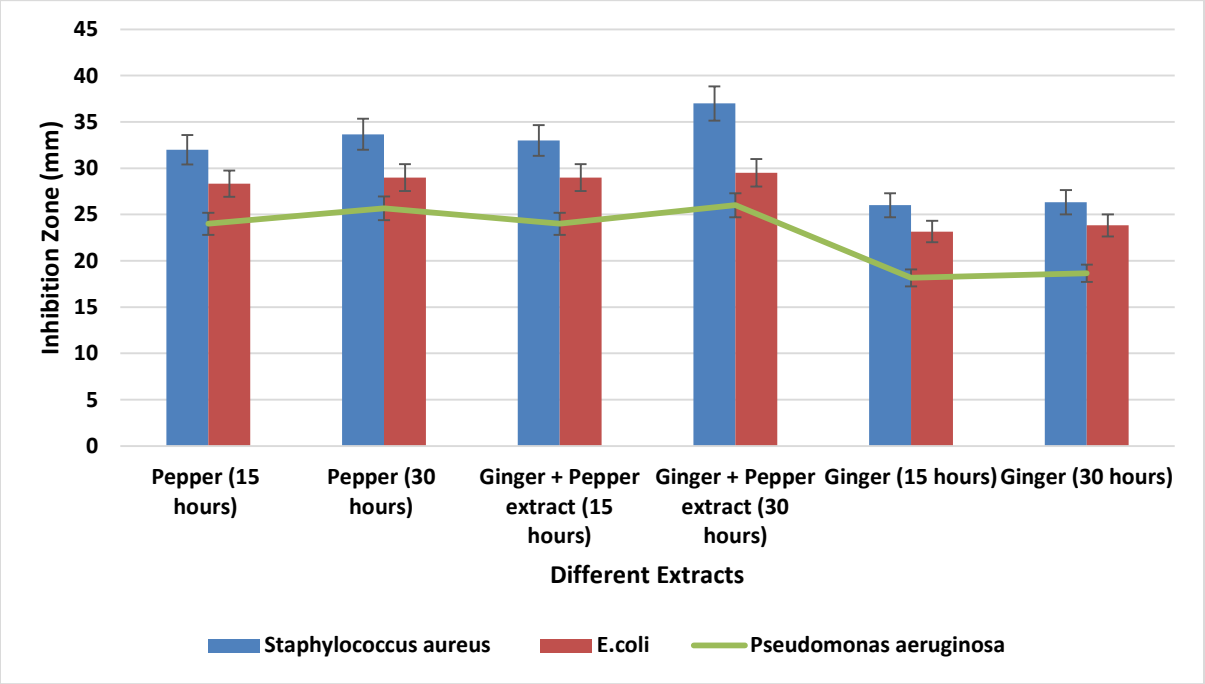

Figure 1. A clustered column of the inhibition zones(mm) of various extracts against the three microbes. The Line in the chart show the level of inhibition exhibited by the P. aeruginosa. The combination of the two extracts show antimicrobial synergy.

Differential Comparative Antibacterial Potency

An analysis of the inhibition zones showed that the mixture of the ginger and pepper had the highest zone of inhibition. The analysis of the three microorganisms showed that the *Staphylococcus aureus* was the most inhibited by the three combinations of the extract. The *Pseudomonas aeruginosa* was the least inhibited by the extracts. The ginger extract showed the least effect of antibacterial effect on the *P. aeruginosa* but it was surprising that its effect on *E.*

coli was reduced in the combined extract with pepper (Figure 2).

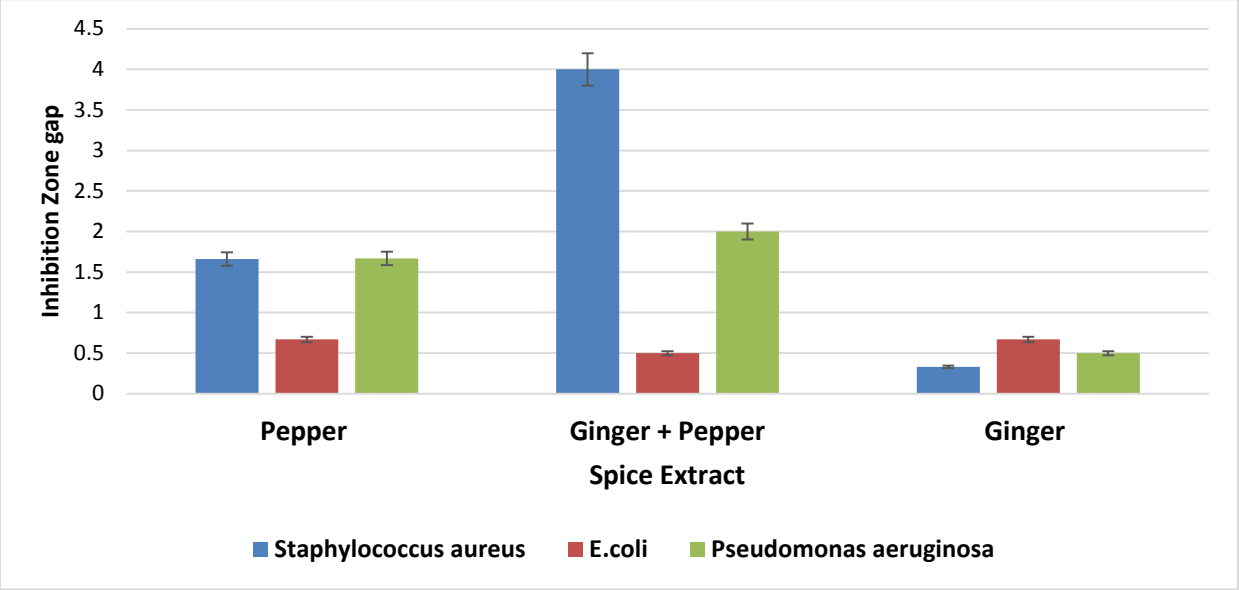

Figure 2. A clustered column chart showing different synergetic effect of spice extracts as differential antibacterial agents. The E. coli had a negative effect on the combined ginger and pepper extracts.

DISCUSSIONS

The study shows that the spices studied possess significant antimicrobial properties, with a greater antimicrobial efficacy when used synergistically. This might be due to resultant effect of the active agents in the spices. The *Capsicum annum* extract tested showed antibacterial properties on both gram positive and gram negative bacteria used in the investigation. This is in conformity with previous studies carried out by Soetarno et al,.1997. This means tropical *capsicum annum* is a potential antibacterial agent. Furthermore, these antimicrobial activities shown by ginger and chilli pepper rhymes with later findings of others (Hara et al, 1998). The antibacterial potential of ginger and chili pepper is perhaps due to compounds like flavonoids, gingerols, volatile oils, terpenoid and volatile oils which are secondary metabolites produced by the plants in response to stress and have protective properties were dissolved in organic compounds according to Jones, et al, 1997 and Avatope et al., 2000.

Pepper extracts

The sharp taste of capsicum peppers is due to the mixture of seven related alkaloids of which capsaicin is the most prevalent. Capsaicinoids are mainly found in the seed and placental area of the chilli pepper (Dong et al,. 2000). The substances' responsible for the pungency are the capsaicinoids alkaloids. They are characterized by high biological activity and their pharmacological activities. The use of the whole fruit in these studies could have contributed more to the antibacterial potency demonstrated both when used independently and when used in combination with ginger.

Ginger extracts

Comparatively, the ginger extracts were less active against *Pseudomonas aeruginosa* which is naturally resistant to antibacterial agents (Walker and Edward et al, 1999). However, this results are in accordance with the results obtained in this study since in the replicates most of zones of inhibition remained constant after 15 hours while zones of inhibition for the other bacteria were increasing. This is probably due to high calorific value per 100g of ginger compared to pepper which seem to feed the microbes rather than kill them. (Figure 3). In similar study by Bonjar et al., 2004, the methanol extracts of ginger rhizomes were active against all gram-positive bacteria and gram-negative bacteria in accordance to the results obtained in this study. The active compounds in chilli pepper and ginger, which have antibacterial activity dissolved in methanol during extraction were actually demonstrated to not only inhibit growth of microbes but also kill the tested bacteria species (Jones, et al, 1997). Greater antibacterial efficacy is seen when the extracts are used synergistically due to probably non-neutralizing resultant effect of the active agents in the plant material. Gram negative bacteria are more resistant to antibiotics than gram positive. The resistance is due to their differences in their cell wall composition. In gram negative the outer membrane acts as great barrier to many environmental substances including antibiotics (Tortora *et al,* 2001).

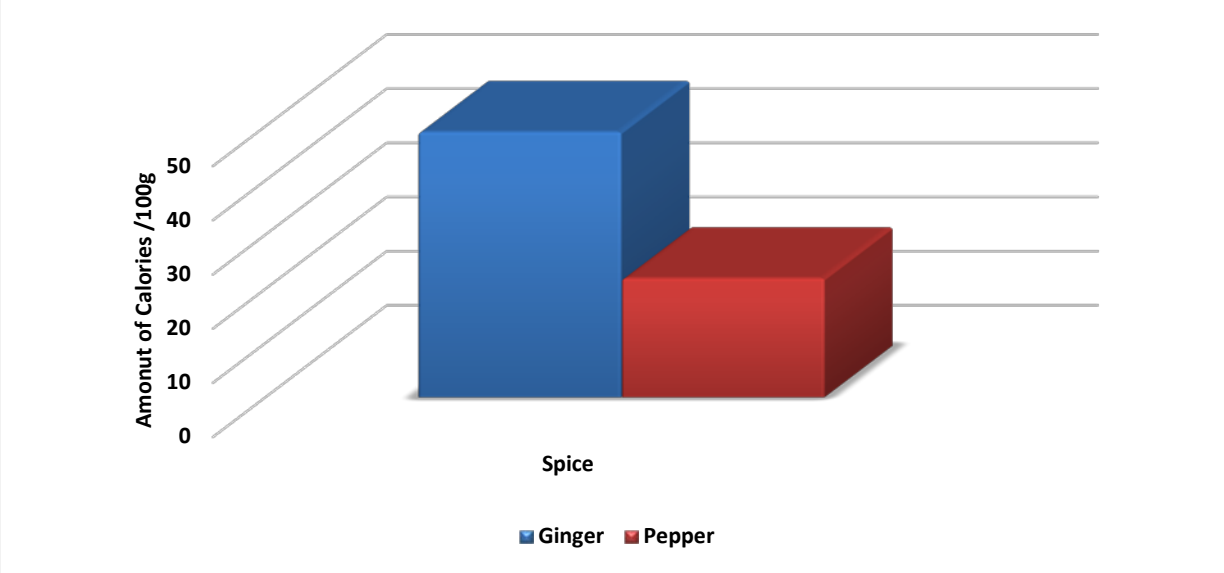

Figure 3. The differences in calorific values of the two spices in per 100 g. (Source, Periodic Table of vegetables, Goodfellow. Cambridge Science Park, USA).

CONCLUSION

In conclusion, the results have provided the justification for therapeutic potential of spices and also opened a new field of studying positive synergetic influences of spices. There might be some cases of negative synergetic effects of some spices. The use of the combined mixtures of spices as supplementary or alternative medicine can be an avenue to reduce the problem of drug resistance, side effects of medicine and reduce cost of treatment. This is feasible even in developing countries who are experiencing high degree of land fragmentation, high population, low immunity levels due to the HIV and AIDS epidemic. This furthermore more welcome in the growing urbanization of agricultural lands since ginger and chili pepper are natural spices which are readily available all year round in markets. They are also comparatively cheap and fairy nonperishable, hence requiring non-sophisticated post-harvest management procedures.

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